(12) UK Patent Application (19) GB (11) 2 321 852 (13) A

(43) Date of A Publication 12.08.1998

(21) Application No 9802821.0

(22) Date of Filing 10.02.1998

(30) Priority Data
(31) 09041658 (32) 10.02.1997 (33) JP
(31) 10038066 (32) 04.02.1998

(71) Applicant(s)
Rohto Pharmaceutical Co Ltd
(Incorporated in Japan)
8-1, Tatsuminishi 1-chome, Ikuno-ku, Osaka-shi,
Osaka 544-0012, Japan

(72) Inventor(s)
Kenji Hamada
Yoko Makino

(74) Agent and/or Address for Service
Mathys & Squire
100 Grays Inn Road, LONDON, WC1X 8AL,
United Kingdom

(51) INT CL[6]
A61K 7/06 38/18 , C07K 14/50

(52) UK CL (Edition P)
A5B BFC

(56) Documents Cited
EP 0455422 A2 WO 95/24414 A1 WO 93/00079 A1
US 4832946 A

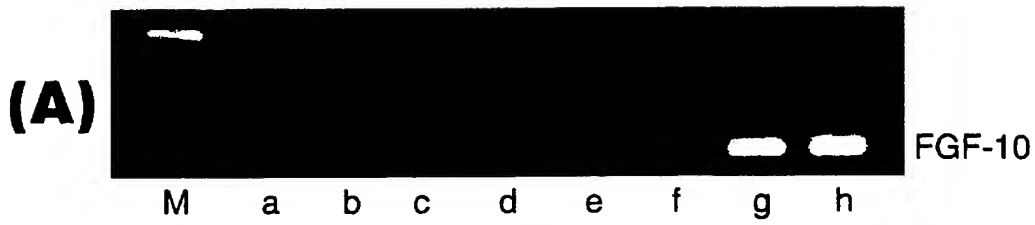
(58) Field of Search
INT CL[6] A61K 7/06 38/18 , C07K 14/50
ON-LINE: STNEXPRESS (INDEX PHARMACOLOGY),
WPI, JAPIO

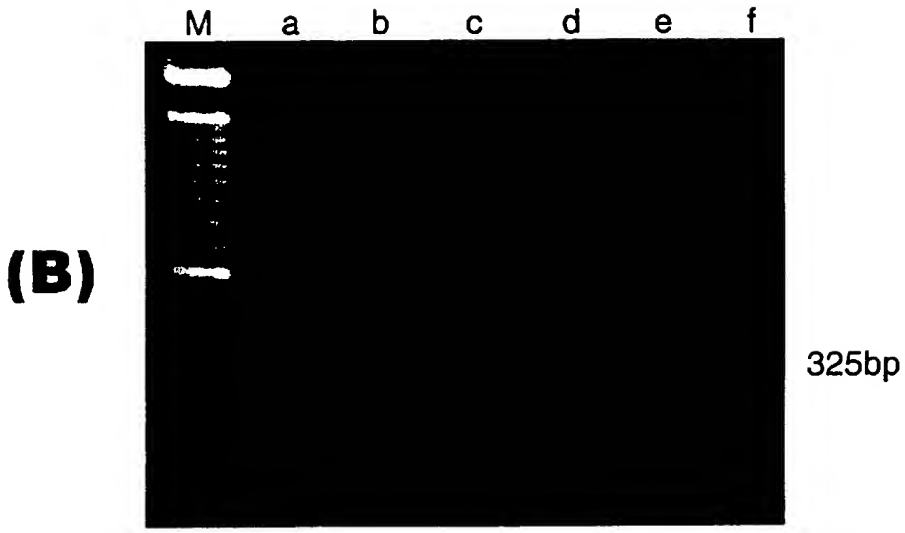
(54) Abstract Title
Effect of Fibroblast Growth Factor 10 (FGF-10) on Hair Cells.

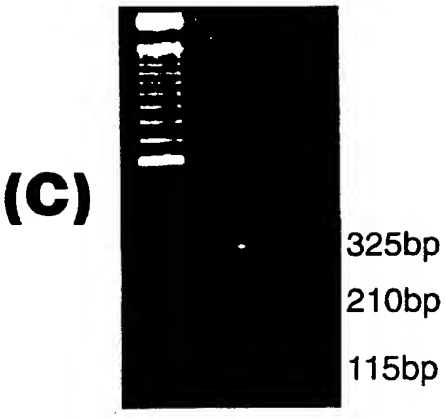
(57) The growth promoting effect of FGF-10 on hair follicular tissues prepared from isolated hair follicles is demonstrated. Thus the use of FGF-10 as the effective ingredient in a hair growth agent is claimed. The FGF-10 used can be prepared by standard protein purification methods or by using recombinant DNA techniques. If the latter the base sequence used for expressing FGF-10 is that of SEQ ID NO 1 or any encoding the protein having substantially the amino acid sequence of SEQ ID NO 2.

GB 2 321 852 A

FIG. 1







HAIR GROWING AGENT

The present invention relates to a hair growing agent comprising FGF-10, belonging to the fibroblast growth factor (FGF) family, as an effective ingredient.

Hitherto hair growing agents have been developed with the promotion of proliferation of trichoepithelial cells (such as matrix cells) as the main purpose including the elevation of metabolism of perifollicular tissues and the enhancement of bloodstream in capillaries of said tissues using "cellular activation" or "activation" as markers. As a result, extracts of placenta, ginseng and swertia herb, pantothenic acid and its derivatives, vitamin E and its derivatives, capsicum tincture, nicotinic acid derivatives, etc. have been used in hair growing agents as effective ingredients showing cell activation activity and bloodstream promoting activity.

However, cellular activators, activators, etc. with highly wide applicability that are expected to only activate trichoepithelial cells, which will become hair constituting cells, must frequently be administered in high doses because of limitations in their applicable amounts, resulting in bringing about many inevitable defects (pungency, etc.).

In follicular tissues involved in hair growth, especially their lower portions, undifferentiated matrix cells of the epithelium are present surrounding the border of hair dermal papilla cells of the mesenchyme and basement membrane. The normal hair growth is assumed to be brought about by proliferation and differentiation of matrix cells due to the interaction between these tissues. In other words, it is assumed that the proliferation and differentiation of matrix cells governed by hair dermal papilla cells lead to the formation of hair shaft including pith, cortex of hair shaft, cuticula pili, etc. and inner root sheath, and the upward movement of matrix cells due to their active proliferation and differentiation results in the hair extension.

For example, it has b en reported that when hair dermal papilla or cultured hair

dermal papilla cells are embedded between epidermis and dermis of plantar skin of mouse and rat having no hair follicl and transferred into the implantation b d, the plantar skin, which does originally not induce hair follicles, envelopes the hair papilla and cultured hair dermal papilla cells to induce the formation of hair follicles [Kobayashi, K., et al., J. Invest. Dermatol. 92, 278-282 (1989), Reynolds, A., et al., Ann. NY Acad. Aci. 642, 226-242 (1991)]. It has also been reported that the induced hair follicles are prescribed by the kind and size of the hair dermal papilla embedded beforehand [Jahoda, C., Development 115, 1103-1109 (1992)].

On the other hand, as to the interaction between hair follicular tissues, there has been reported the androgen-dependent hair growth. For example, proliferation of beard epithelial cells in the presence of androgen-dependent beard dermal papilla cells is completely suppressed by a neutralizing antibody against the insulin-like growth factor-I (IGF-I). Further, IGF-I mRNA is expressed in beard dermal papilla cells and this expression is controlled by androgens. These facts has been proved that at least IGF-I is one of paracrine growth factors directly acting on neighbouring cells with being secreted in the interaction between androgen-dependent hair follicle tissues derived from hair dermal papilla cells [Itami, S., et al., Biochem. Biophys. Res. Commun. 212, 988-994 (1995), Itami, S. et al., Hair Research for the Next Millenium 297-302 (1996) Elsevier Science].

At present, although mesenchymal hair dermal papilla cells unmistakably play a key role in hair growth, there still remain many unclear points to be elucidated concerning mechanisms of interaction between hair follicle tissues.

An object of the present invention is to provide a paracrine factor capable of participating in interactions between hair follicle tissues, particularly a paracrine factor expressed in hair dermal papilla cells, which plays a key role in hair growth. Based on this, another object of the present invention is to provide a hair growing agent comprising this factor as an ffective ingredient.

Hair dermal papilla cells are assum d to play important roles in not only the

induction of differentiation from skin tissues to follicular tissues but also the maintenanc of hair cycle as well as the differentiation and prolif ration of matrix cells in the anagen phase. In addition, it is assumed that signals emitted from hair dermal papilla cells are profoundly involved in roles of these cells. Therefore, it can be said that the determination of these signals is very important for elucidating mechanisms of interactions between hair follicular tissues.

In order to attain the above-described objects, the present inventors have intensively studied and, as a result, elucidated that a factor known as the fibroblast growth factor-10 (FGF-10) [Yamasaki, M. et al., J. Biol. Chem. 271, 15918-15921 (1996), Emoto et al., Abstracts 163 (1996), Joint Annual Assembly of the 69th General Meeting of The Japanese Biochemical Society and the 19th General Meeting of The Molecular Biology Society of Japan] is expressed in human hair dermal papilla cells and promotes growth of hair follicles. Thus, the present inventors have found that FGF-10 would be useful as a hair growing agent, thereby achieving the present invention.

FGF-10 is the tenth factor belonging to the FGF family which was isolated from a rat embryonic cDNA library by PCR using a partial region of the highly homologous amino acid sequence retained among FGF family members as primer [Yamasaki, M., et al., J. Biol. Chem. 271, 15918-15921 (1996)]. As to other members belonging to the FGF family, there have hitherto been reported their applications for melanin synthesis inhibitor (JP-A-Hei 7-304686), liver function ameliorator (JP-A-Hei 6-345666), and further skin cosmetic (JP-A-Hei 4-187613). In addition, there have been reported applications for the treatment and prevention of alopecia (JP-A-Hei 4-224522) and hair tonic (growing) cosmetic as well as canities amelioration cosmetic (JP-A-Hei 8-208440).

On the other hand, as to FGF-10, there have been reported its specific expressions in posterior pituitary, first cervical vertebrat , duodenum, lung, sacral and coccygeal segments of the spinal cord of rat embryos, and in lung of adult rats [Yamasaki, M. et al., J. Biol. Ch m. 271, 15918-15921 (1996)]. Also, it has been

reported that FGF-10 has activity to proliferate epithelial cells. However, nothing has been known at all about the relationship betw en human hair tissue, sp cially hair dermal papilla cells governing hair growth, and FGF-10.

The present inventors isolated hair dermal papilla cells, epidermal keratinocytes, and outer root sheath cells, carried out RT-PCR with total RNA in these cells, and, as a result, elucidated for the first time that FGF-10 was expressed in hair dermal papilla cells which play a central role in the hair growth. Further, the present inventors examined the growth-promoting effect of FGF-10 on hair follicular tissues prepared from isolated hair follicles and found for the first time that FGF-10 actually promoted the growth of hair follicles.

The present invention relates to a paracrine factor "FGF-10" capable of promoting the growth of hair follicles, more specifically to a hair growing agent comprising FGF-10 as an effective ingredient.

Fig. 1 A is the electrophoretic pattern showing the expression of FGF-10 in the frontal hair dermal papilla cells, epidermal keratinocytes, outer root sheath cells, and beard dermal papilla cells, detected by RT-PCR. Fig. 1 B is the electrophoretic pattern showing the expression of FGF-10 in hair dermal papilla cells derived from different subjects detected by RT-PCR. Fig. 1 C is the electrophoretic pattern of FGF-10 treated with restriction enzyme.

FGF-10 used as an effective ingredient of the hair growing agent of the present invention can be prepared from tissues or cells of warm-blooded animals by the standard protein purification method, and also using recombinant DNA techniques. The natural FGF-10 can be isolated and purified, for example, by suitable combinations of widely used chromatographic techniques, salting out, solvent extraction, ultrafiltration concentration, recrystallization, centrifugation, distillation, etc. In the case of preparation of FGF-10 using recombinant techniques, as the base sequence used for expressing FGF-10, in addition to thos describ d in SEQ ID NO: 1, any bas s quences encoding the protein having substantially the same

amino acid sequence described in SEQ ID NO: 2 can be used. The recombinant FGF-10 can be prepared, for example, by transforming host cells with th recombinant expression vector in which a DNA comprising base sequences encoding the total or partial amino acid sequences of FGF-10 has been cloned, culturing the transformant thus obtained in a suitable medium and under suitable culture conditions, extracting and purifying the product from said transformant. There is no limitation in the type of expression vectors, so far as they can replicate, proliferate, transcribe and translate in hosts. As hosts to be transformed with the vector, for example, Escherichia coli, yeast, insect cells, mammalian cells, etc. can be used.

Also, in the present invention, it is possible to use modified FGF-10 peptides. Modified FGF-10 peptides may be produced naturally or by the post-translational modification. Also, they can be prepared using DNAs modified by genetic engineering, for example, site-specific mutagenesis [Methods in Enzymology 154, 350, 367-382 (1987), ibid. 100, 468 (1983), Nucleic Acids Research 12, 9441 (1984)], etc., and chemical synthetic methods such as phosphate triester method and phosphate amidite method [J. Am. Chem. Soc. 89, 4801 (1967), ibid. 91, 3350 (1969), Science 150, 178 (1968), Tetrahedron Lett. 22, 1859 (1981), ibid. 24, 245 (1983)]. Furthermore, it is possible to prepare them by suitable combinations of these methods.

FGF-10 of the present invention may be used alone or in combination with other ingredients as the hair growing agent. Other ingredients may be exemplified by cellular activators, circulation enhancers, anti-androgens, etc., more specifically, pantothenic acid and its derivatives, placenta extract, photoelements, ginseng extract, biotin, mononitroguaiacol, carpronium chloride, vitamin E and its derivatives, swertia herb extract, capsicum tincture, cephalanthin, nicotinic acid and its derivatives, estradiol, ethynilestradiol, landic acid, minoxidil and its homologues and derivatives, 5α reductase inhibitor, 12-tetradecanoylphorbol-13-acetate, and crude drugs such as polygonati rhizoma, Uncaria rhyncophylla, henna, glycyrrhiza, etc., but not limited to them. It is

possible to use th se ingredients in combination of more than two of them. These combinations can be made in any ratios so far as they can effectively promote hair growth or prevent alopecia.

In the case of topical application of FGF-10 of the present invention, it can be administered together with pharmaceutically and topically acceptable carriers or media usually used for topical compositions. For example, FGF-10 of the present invention may be used in combination with aqueous ingredient, powder, surfactant, medicinal oil, humectant, alcohol, pH regulator, antiseptic, antioxidant, thickener, vitamins, sebolytic, disinfectant, keratolytic, perfume, pigment, etc., which are usually used in cosmetics, medicines, and medicines for topical application. In the case of topical application, for the more effective use of FGF-10, it can be used together with known DDS carriers such as liposome.

Dosage of the hair growing agent of the present invention is usually about 0.001 to about 100 μg/day/cm^2, and applicable concentration is 0.0001-0.1 w/v%, preferably about 0.01 to about 10 μg/day/cm^2, and 0.0005-0.05 w/v%. Daily dosage is appropriately selected according to the conditions of scalp, etc., and the necessary dosage can be administered divided in one to several portions per day.

The hair growing agent of the present invention can be formulated into a pharmaceutical preparation or a cosmetic, such as cream, lotion, ointment, gel, shampoo, aerosol, etc.

Since FGF-10 of the present invention is considered to exhibit an alopecia treating and preventing effect as well as a hair growing effect, the hair growing agent of the present invention can be used as an alopecia treating agent and an alopecia preventing agent. Further, considering that it is of the biological origin and highly safe, it can be used as hair growing/hair tonic cosmetic and alopecia preventive cosmetic.

According to the present inv ntion, it was elucidat d that FGF-10 is expressed in

hair dermal papilla cells and is a paracrine factor that plays a key role in th hair growth. Based on this finding, the present invention provid s the hair growing agent comprising FGF-10 as an effective ingredient.

The following Examples illustrate the present invention in detail, but are not construed to limit the scope of the invention.

EXAMPLE 1

Isolation of hair dermal papilla cells

Hair dermal papilla cells were isolated from full-thickness skin obtained at the plastic surgery operation according to the usual method (Messenger, A. G., Br. J. Dermatology 110, 685-689, 1984). The following operations were all carried out under stereoscopic microscope in a clean bench.

Hair follicles with peripheral tissues were selectively excised from full-thickness skin using a 21 G needle. From excised hair follicles were removed adhering peripheral tissues, and only hair follicle bulbs were cut off. Hair follicle bulbs were dissected, and only hair dermal papillae were excised and used for primary culture.

Hair dermal papillae thus obtained were placed in a cell density of 4 to 5 cells per a 35-mm culture dish, and cultured at 37°C in a 5% CO_2 atmosphere under humidified conditions in the 199 medium (Gibco BRL) containing penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mmol/ml), and 20% fetal calf serum. The medium was exchanged every 3 day after hair dermal papillae adhered to the bottom surface of the dish. "Epidercell NHEK(F)" (Kurabo) was used as epidermal keratinocytes and cultured in "HuMedia-KG2 medium" (Kurabo). Outer root sheath cells were isolated by the usual method and cultured in "MCDB153 medium" (Sigma).

The above-describ d c lls were all subcultured after th y r ach subconfluenc using PBS(-) containing 0.05% trypsin and 0.02% EDTA according to the usual

method. In th se experiments, cells subcultured 3 to 4 generations were used.

EXAMPLE 2

RT-PCR (reverse transcriptase-polymerase chain reaction)

1. Purification of total RNA

Purification of total RNA from cultured hair dermal papilla cells can be performed according to the usual method. Specifically, it was purified using ISOGEN (Nippon Gene) according to the "guanidinium thiocyanate-phenol-chloroform method" (Chomczynski, P. et al., Anal. Biochem. 162, 156-159, 1987). In addition, total RNAs from epidermal keratinocytes and outer root sheath cells were similarly prepared.

2. Synthesis of single-stranded cDNA (reverse transcriptase reaction)

Synthesis of single-stranded cDNA from purified total RNA can be performed according to the usual method. Specifically, a tube containing a mixture (100 μl) comprising "2.5 M random hexamers, 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), each 1 mM deoxynucleotide triphosphate (dNTP), 1.0 U/μl ribonuclease inhibitor (those described above from Takara Shuzo), 2.5 U/μl M-MLV reverse transcriptase (Gibco BRL) and 2 μg of total RNA was placed in a thermal cycler after denaturation, and the synthetic reaction was performed according to the following program. That is, the reaction was carried out "at 25°C for 15 min, at 42°C for 45 min, at 99°C for 5 min, and at 4°C for 10 min" for one cycle.

3. PCR (polymerase chain reaction)

PCR can be performed according to the usual method. Specifically, a PCR mixture was prepared by adding 5 μl of a sample solution resulted from the above-described reverse transcriptase reaction to a solution (total 20 μl) placed in a tube containing 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.025 U/μl r combinant Taq

DNA polymerase (Takara Shuzo), and each 0.5 μM primer. As prim rs, 5'-ACCAACTCTTCTTCCTCCTC-3' (SEQ ID NO: 3) and 5'-CCTCTATCCTCTCCTTCAGC-3' (SEQ ID NO: 4) were used. To this mixture was added one drop of mineral oil (Perkin-Elmer), and the resulting mixture was placed in a thermal cycler. PCR was performed "at 94°C for 1 min" for 1 cycle, "at 94°C for 1 min, at 60°C for 1 min, and at 72°C for 1 min" for 35 cycles, "at 72°C for 10 min" for 1 cycle, and "at 4°C for 10 min" for 1 cycle. After the PCR reaction, amplified products were visualized by electrophoresis on 2% agarose gel containing ethydium bromide (0.5 μg/ml).

As a result, the expression of FGF-10 was recognized in mesenchymal hair dermal papilla cells (a, b, g, and h in Fig. 1 A). In Fig. 1 A, "M" represents 100-bp molecular weight marker, "a" and "b" show the results obtained with frontal hair dermal papilla cells, "c" and "d" with epidermal keratinocytes, "e" and "f" with outer root sheath cells, and "g" and "h" with beard dermal papilla cells.

In addition, RT-PCR was similarly performed using hair dermal papilla cells derived from different subjects (Fig. 1 B). In Fig. 1 B, "M" represents 100-bp molecular weight marker, "a" through "f" show the experimental results with hair dermal papilla cells derived from different subjects. In hair dermal papilla cells derived from all different subjects and used in this experiment, a band was detected at the position corresponding to 325 bp. The base sequence of amplified fragment is shown in SEQ ID NO: 5.

In order to confirm whether the thus-detected band is FGF-10 band, the restriction enzyme digestion analysis was carried out. Specifically, a single band which was confirmed by electrophoresis was excised from the gel, and DNA was recovered therefrom using "The GENECLEAN II Kit" (BIO 101). The PCR-amplified product thus recovered was digested with restriction enzyme ScaI (Takara Shuzo), and digests were visualized by the abov -described electrophoresis. As a result, bands in size of 210 bp and 115 bp expectabl from the sequenc of FGF-10 were detect d (Fig. 1 C). In Fig. 1 C, the left lan shows th lectrophor tic pattern of

a 100 bp molecular weight marker (BRL Life Technology), the middle lane shows that of the sample without Sca I added, and the right lane shows that of the Sca I-digested sample.

EXAMPLE 3

Preparation of recombinant FGF-10

For preparing recombinant FGF-10, FGF-10 cDNA was prepared by PCR. Specifically, a PCR mixture (50 μl) was prepared by mixing 5 μl of 10 x Pfu buffer (Stratagene), 0.4 mM dNTP (Takara Shuzo), 5U of Pfu DNA polymerase (Stratagene), each 0.5 μM primer, and 5 μl of a sample solution resulted from the reverse transcriptase reaction prepared in Example 2 (2). As primers, 5'-AAGCTTATGTGGAAATGGATACTG-3' (SEQ ID NO: 6) and 5'-AAGCTTCTATGAGTGTACCACCAT-3' (SEQ ID NO: 7) were used. PCR was performed "at 94°C for 1 min" for 1 cycle, "at 94°C for 1 min, at 56°C for 1 min, and 72°C for 2 min" for 35 cycles, "at 72°C for 10 min" for 1 cycle, and "at 4°C for 10 min" for 1 cycle. The resulting PCR products were electrophoresed on agarose gel. Then, the product of a band in size of 640 bp was purified from the gel and digested with the restriction enzyme HindIII. The resulting HindIII fragment containing the full coding region of human FGF-10 was subcloned into the HindIII site of plasmid pRc/CMW (Invitogen) to give plasmid pRc/CMV-hFGF10. COS-1 cells (American Type Culture Collection) were inoculated in DMEM (Gibco BRL) supplemented with 10% fetal calf serum to give a cell density of 2 x 105 cells per 60 mm dish 24 hours before transfection. Five μg of pRc/CMV-hFGF10 containing the recombinant DNA molecule and pRc/CMV (control) having no recombinant DNA molecule introduced therein were employed for transfection by the calcium phosphate precipitation method [Graham et al., Virology 52, 456 (1973)]. After 24-hour culture, the medium was replaced with DMEM supplemented with 0.1% fetal calf serum to culture for additional 40 hours. Then, the transfected COS-1 cells and the culture supernatant were harvested. The cells were rinsed twice with PBS, were suspended in 20 mM Tris-HCl (pH 7.6) containing 1 M NaCl, and were disrupted by sonication. The sup rnatant was collected by c ntrifugation at 4°C

at 15,000 g for 15 min. The resulting supernatant and the cultur supernatant were concentrated by ultrafiltration (molecular cut-off, 10,000) to obtain filtrate A (derived from COS-1 cell transfected with pRc/CMV-hFGF10 containing the recombinant DNA molecule) and filtrate B (derived from COS-1 transfected with pRc/CMV containing no recombinant DNA molecule as a control).

EXAMPLE 4

Isolation of human hair follicles

Human hair follicles were isolated from scalp biopsies from normal subjects obtained at the plastic surgery operation according to the usual method [Philpott, M.P. et al., J. Cell Sci. 97, 463-471 (1990)]. The following operations were all carried out under stereoscopic microscope in a clean bench.

These strips were cut into small pieces not more than 5 mm wide. The epidermis and upper dermis were separated from the underlying fat layer containing hair follicle bulbs. The fat tissues containing hair follicle bulbs were then placed in a culture medium, and the hair follicles were isolated under stereomicroscope (Leica WILD MZ8) by gently pulling them out of the fat layer using forceps.

EXAMPLE 5

Hair follicle organ culture and measurements of hair growth

Individual, freshly isolated hair follicles as described in Example 4 were maintained at 37°C in an atmosphere of 5% CO_2/95% air in each well of 12-well plates (CORNING Coster) for 5 days. The filtrates A and B obtained in Example 3 were respectively diluted 10,000-fold and 100,000-fold with a control medium containing William's E medium (Gibco BRL) supplemented with 2 mM L-glutamine, (Nacalai tesque) insulin (10 μg/ml)(Sigma, the following components were also purchased from Sigma), transferrin (10 μg/ml), hydrocortisone (10 ng/ml), sodium selenite (10 ng/ml), penicillin (50 μg/ml), and streptomycin (50 IU/ml) to prepare the test culture media. The hair follicl organ culture was made in the

thus-prepared test media to evaluate their growth. The growth was defin d as an increase in l ngth of the whole follicle cultured for a predetermined period of time by measuring under observation with an inverted microscope (OLYMPUS IX70) equipped with an eyepiece measuring graticule. As a result, the hair follicle cultured in the medium in which filtrate A was diluted 100,000-fold showed about 1.3 mm extension. The hair follicle growth in this culture was promoted by about 15% higher than that in the culture containing filtrate B with the same dilution. Further, about 1.4 mm extension was observed for the hair follicle cultured in the medium containing 10,000-fold dilution of filtrate A, which was about 26% longer than that cultured in the medium containing filtrate B with the same dilution.

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT: Rohto Pharmaceutical Co., Ltd.

(ii) TITLE OF INVENTION: Hair growing Agent

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE:
- (B) STREET:
- (C) CITY:
- (D) STATE:
- (E) COUNTRY:
- (F) ZIP:

(v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Diskette - 5.25 inch, 1 Mb storage.
- (B) COMPUTER: NEC PC-9801 Series
- (C) OPERATING SYSTEM: MS-DOS Ver3.30 or Later
- (D) SOFTWARE:

(ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE:
- (B) TELEFAX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

```
ATG TGG AAA TGG ATA CTG ACA CAT TGT GCC TCA GCC TTT CCC CAC CTG       48
Met Trp Lys Trp Ile Leu Thr His Cys Ala Ser Ala Phe Pro His Leu
1               5                  10                  15
CCC GGC TGC TGC TGC TGC TGC TTT TTG TTG CTG TTC TTG GTG TCT TCC       96
Pro Gly Cys Cys Cys Cys Cys Phe Leu Leu Leu Phe Leu Val Ser Ser
            20                  25                  30
GTC CCT GTC ACC TGC CAA GCC CTT GGT CAG GAC ATG GTG TCA CCA GAG      144
Val Pro Val Thr Cys Gln Ala Leu Gly Gln Asp Met Val Ser Pro Glu
        35                  40                  45
GCC ACC AAC TCT TCT TCC TCC TCC TTC TCC TCT CCT TCC AGC GCG GGA      192
Ala Thr Asn Ser Ser Ser Ser Ser Phe Ser Ser Pro Ser Ser Ala Gly
    50                  55                  60
AGG CAT GTG CGG AGC TAC AAT CAC CTT CAA GGA GAT GTC CGC TGG AGA      240
Arg His Val Arg Ser Tyr Asn His Leu Gln Gly Asp Val Arg Trp Arg
65                  70                  75                  80
AAG CTA TTC TCT TTC ACC AAG TAC TTT CTC AAG ATT GAG AAG AAC GGG      288
Lys Leu Phe Ser Phe Thr Lys Tyr Phe Leu Lys Ile Glu Lys Asn Gly
                85                  90                  95
AAG GTC AGC GGG ACC AAG AAG GAG AAC TGC CCG TAC AGC ATC CTG GAG      336
Lys Val Ser Gly Thr Lys Lys Glu Asn Cys Pro Tyr Ser Ile Leu Glu
            100                 105                 110
ATA ACA TCA GTA GAA ATC GGA GTT GTT GCC GTC AAA GCC ATT AAC AGC      384
Ile Thr Ser Val Glu Ile Gly Val Val Ala Val Lys Ala Ile Asn Ser
        115                 120                 125
AAC TAT TAC TTA GCC ATG AAC AAG AAG GGG AAA CTC TAT GGC TCA AAA      432
Asn Tyr Tyr Leu Ala Met Asn Lys Lys Gly Lys Leu Tyr Gly Ser Lys
    130                 135                 140
GAA TTT AAC AAT GAC TGT AAG CTG AAG GAG AGG ATA GAG GAA AAT GGA      480
Glu Phe Asn Asn Asp Cys Lys Leu Lys Glu Arg Ile Glu Glu Asn Gly
145                 150                 155                 160
TAC AAT ACC TAT GCA TCA TTT AAC TGG CAG CAT AAT GGG AGG CAA ATG      528
Tyr Asn Thr Tyr Ala Ser Phe Asn Trp Gln His Asn Gly Arg Gln Met
                165                 170                 175
TAT GTG GCA TTG AAT GGA AAA GGA GCT CCA AGG AGA GGA CAC AAA ACA      576
Tyr Val Ala Leu Asn Gly Lys Gly Ala Pro Arg Arg Gly His Lys Thr
            180                 185                 190
```

```
CGA AGG AAA AAC ACC TCT GCT CAC TTT CTT CCA ATG GTG GTA CAC TCA      624
Arg Arg Lys Asn Thr Ser Ala His Phe L u Pro Met Val Val His Ser
        195                 200                 205
TAG                                                                 627
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

```
Met Trp Lys Trp Ile Leu Thr His Cys Ala Ser Ala Phe Pro His Leu
1               5                  10                  15
Pro Gly Cys Cys Cys Cys Cys Phe Leu Leu Leu Phe Leu Val Ser Ser
            20                  25                  30
Val Pro Val Thr Cys Gln Ala Leu Gly Gln Asp Met Val Ser Pro Glu
        35                  40                  45
Ala Thr Asn Ser Ser Ser Ser Ser Phe Ser Ser Pro Ser Ser Ala Gly
    50                  55                  60
Arg His Val Arg Ser Tyr Asn His Leu Gln Gly Asp Val Arg Trp Arg
65                  70                  75                  80
Lys Leu Phe Ser Phe Thr Lys Tyr Phe Leu Lys Ile Glu Lys Asn Gly
                85                  90                  95
Lys Val Ser Gly Thr Lys Lys Glu Asn Cys Pro Tyr Ser Ile Leu Glu
            100                 105                 110
Ile Thr Ser Val Glu Ile Gly Val Val Ala Val Lys Ala Ile Asn Ser
        115                 120                 125
Asn Tyr Tyr Leu Ala Met Asn Lys Lys Gly Lys Leu Tyr Gly Ser Lys
    130                 135                 140
Glu Phe Asn Asn Asp Cys Lys Leu Lys Glu Arg Ile Glu Glu Asn Gly
145                 150                 155                 160
Tyr Asn Thr Tyr Ala Ser Phe Asn Trp Gln His Asn Gly Arg Gln Met
                165                 170                 175
Tyr Val Ala Leu Asn Gly Lys Gly Ala Pro Arg Arg Gly His Lys Thr
            180                 185                 190
Arg Arg Lys Asn Thr Ser Ala His Phe Leu Pro Met Val Val His Ser
        195                 200                 205
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid, synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

ACCAACTCTT CTTCCTCCTC 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid, synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4
CCTCTATCCT CTCCTTCAGC 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

ACCAACTCTT CTTCCTCCTC CTTCTCCTCT CCTTCCAGCG CGGGAAGGCA TGTGCGGAGC 60
TACAATCACC TTCAAGGAGA TGTCCGCTGG AGAAAGCTAT TCTCTTTCAC CAAGTACTTT 120
CTCAAGATTG AGAAGAACGG GAAGGTCAGC GGGACCAAGA AGGAGAACTG CCCGTACAGC 180
ATCCTGGAGA TAACATCAGT AGAAATCGGA GTTGTTGCCG TCAAAGCCAT TAACAGCAAC 240
TATTACTTAG CCATGAACAA GAAGGGGAAA CTCTATGGCT CAAAAGAATT TAACAATGAC 300
TGTAAGCTGA AGGAGAGGAT AGAGG 325

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid, synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

AAGCTTATGT GGAAATGGAT ACTG 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESSS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid, synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

AAGCTTCTAT GAGTGTACCA CCAT 24

CLAIMS

1. A hair growing agent comprising FGF-10 as an effective ingredient.

2. The hair growing agent according to claim 1, wherein FGF-10 is a recombinant protein.

3. The hair growing agent according to claim 2, wherein FGF-10 has the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2

4. The hair growing agent according to claim 1, which further comprises carriers or media acceptable for topical application.

5. The hair growing agent according to claim 1, wherein FGF-10 is contained in an amount effective for promoting the hair growth.

6. Use of FGF-10 for the preparation of a hair growing agent.

7. A hair growing agent substantially as hereinbefore described and claimed.

The Patent Office

18

Application No: GB 9802821.0 **Examiner:** Simon M. Fortt
Claims searched: 1-7 **Date of search:** 18 May 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P):

Int Cl (Ed.6): A61K 7/06, 38/18, C07K 14/50

Other: On-line: STNEXPRESS (INDEX PHARMACOLOGY), WPI, JAPIO.

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	EP 0 455 422 A2	(MERCK & CO. INC.) whole document, especially p 4, ll 9-12.	
X	WO 95/24414 A1	(HUMAN GENOME SCIENCES INC.) whole document, especially p 1, ll 1-10, p 3, ll 26-31, p 4, l 3-16, p17, ll 17-19.	1-2, 4-7
A	WO 93/00079 A1	(UNIVERSITY OF MIAMI) p 2, ll 3-7, p 6, ll 6-18, examples I, II, claim 10.	
X	US 4 832 946	(GREEN) col. 3, ll 19-42, col. 3 l 64 - col. 4, l 42, examples.	1, 4-7

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.